Do arctic breeding Red Knots (Calidris canutus) accumulate skeletal calcium before egg laying?

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Do arctic-breeding Red Knots (*Calidris canutus*) accumulate skeletal calcium before egg laying?

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Abstract: Earlier studies have indicated that the diet of egg-laying female birds which eat only terrestrial arthropods has to be supplemented with calcium if they are to produce high-quality eggshells without interruption. During egg laying, females of tundra-breeding shorebird species may supplement their diet with fragments of mammalian skeletons, but as an alternative strategy they might store skeletal calcium before egg formation. We examine the possibility of calcium storage on the basis of temporal changes in the ash mass (a good indicator of skeletal mass) of male and female Red Knots (*Calidris canutus islandica*) collected during their stopover in Iceland in May and July, and after arrival on the breeding grounds in northernmost Ellesmere Island, Canada, in late May and early June. Significantly higher ash masses of females than of males, an increase in ash mass of females before the period of egg formation in mid-June in combination with a subsequent decrease, and the notable absence of temporal changes in ash mass of males, lead us to propose that female Red Knots do store skeletal calcium before egg laying. The rate of calcium storage would be 2.3 times higher after arrival on Ellesmere Island than during the stopover in Iceland, but the dietary components through which storage is achieved remain unclear. With an almost 50% change in the skeletal mass of females, Red Knots currently hold the record with respect to skeletal calcium dynamics in free-living egg-laying birds. The stored skeletal mass would allow them to produce at least half the clutch without further calcium intake.

Résumé : Des études antérieures ont démontré que, chez les oiseaux, les femelles pondeuses qui ne mangent que des arthropodes terrestres doivent s'assurer un supplément de calcium pour être capables de produire toujours des coquilles de haute qualité. Durant la ponte, les femelles des espèces d'oiseaux de rivage qui se reproduisent dans la toundra peuvent compléter leur régime par la consommation de fragments de squelettes de mammifères, mais il se peut aussi qu'elles mettent du calcium squelettique en réserve avant la formation de leurs oeufs. Nous examinons ici l'hypothèse du stockage du calcium en observant les changements dans la masse des cendres (un bon indicateur de la masse squelettique) chez des mâles et des femelles du Bécasseau maubèche (*Calidris canutus islandica*) capturés durant leur arrêt en Islande en mai et juillet et après leur arrivée dans les territoires de reproduction dans l'île la plus boréale des Ellesmere, Canada, à la fin de mai et au début de juin. Les femelles ont des masses de cendre significativement plus élevées que celles des mâles, elles subissent une augmentation de la masse de leurs cendres avant la période de formation des oeufs à la mi-juin, puis, une diminution de cette masse, alors que les mâles ne subissent aucun changement temporel décelable de leur masse de cendres; ces phénomènes nous portent à croire que les bécasseaux femelles mettent effectivement du calcium squelettique en réserve avant la ponte. Le taux d'accumulation du calcium semble être 2,3 fois plus élevé après l'arrivée dans l'île d'Ellesmere que durant l'arrêt en Islande, mais il est encore impossible de déterminer quelles sont les ressources utilisées pour la mise en réserve du calcium. La masse squelettique des femelles de ce bécasseau varie de près de 50%, un record en ce qui concerne la dynamique du calcium squelettique chez les oiseaux pondeurs en nature. La masse squelettique accumulée permet probablement aux femelles de produire au moins la moitié de leur couvée sans l'apport additionnel de calcium alimentaire.
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Introduction

A high incidence of lemming teeth and bones in the stomachs of prelaying females and growing chicks of four tundra-breeding sandpiper (*Calidris*) species, and the paucity of such skeletal remains in males, full-grown chicks, and non-laying females (MacLean 1974), strongly suggest that the extraction of calcium from a normal diet of terrestrial arthropods does not suffice for eggshell formation and skeletal growth. The much shorter laying interval (24 h) of tundra-breeding Dunlins (*Calidris alpina*) in northern Alaska, where lemming remains can be found, than the 36 h of Dunlins breeding on Finnish coastal meadows, where lemmings are absent, additionally suggests that ingestion of bone fragments

rich in calcium enhances laying performance (MacLean 1974). That the availability of calcium might conceivably constrain the reproductive performance of many arthropod-eating birds (Graveland and van Gijzen 1994; Barclay 1994) has recently been highlighted by reduced breeding productivity of Great Tits (*Parus major*) resulting from a paucity of landsnails, owing to the leaching of calcium by acid rain (Graveland 1990, 1996; Graveland et al. 1994; Graveland and van der Wal 1996). Under normal conditions, prelaying female tits supplement their diet with fragments of shells of landsnails. If snails are in short supply, eggshells become thinner and show abnormalities.

One of the possible adaptations to alleviate a limiting calcium intake rate during egg laying is storage of calcium in the skeleton prior to laying (see reviews in Simkiss 1967; Hurwitz 1987). This is a feasible option for large ground-living birds such as the Galliformes, but it may be less suitable for small aerial species with a relatively light skeleton and calcium-rich clutches (Graveland 1995). Even with a clutch size limited to four eggs, members of the shorebird families Charadriidae and Scolopacidae are predicted not to be able to store all necessary calcium before egg production (Graveland 1995). However, the paucity of relevant descriptive studies means that this possibility has not been tested.

In the course of our investigations of body mass changes and fat and protein dynamics of Red Knots (*Calidris canutus*) migrating from stopover areas in Iceland to the high arctic breeding grounds in northeast Canada, we collected individuals in different phases of their migratory and reproductive cycle for compositional analyses. Some carcasses were incinerated to obtain ash values as structural size indicators; ash largely consists of skeletal material. That ash mass (25–30% of which is calcium; Grimshaw et al. 1958) was lower in males than in females, and showed interesting breeding-related changes in females independent of linear body size dimensions, hinted at the possibility that these small (130 g) long-distance migrant shorebirds do in fact store calcium before egg laying. Here we review evidence for this suggestion in the hope of refocussing attention on this potentially important nutritional bottleneck during reproduction, especially with regard to avian long-distance migrants (cf. Underhill 1994).

Study areas and methods

Study areas

Our study is concerned with individuals of *Calidris canutus islandica*. This is one of the five currently recognised subspecies of Red Knots (Piersma and Davidson 1992), and one that winters in the estuaries of western Europe (Davidson and Wilson 1992). Most of these birds use the intertidal flats of west Iceland as refuelling areas during northward and southward migration to and from their breeding grounds in northeast Canada and northern Greenland (Morrison 1976; Alerstam et al. 1990, 1992; Morrison and Wilson 1992; Wilson and Morrison 1992; Gudmundsson 1993; Gudmundsson and Gardarsson 1993).

In Iceland, Red Knots were collected upon their arrival from the British Isles as they approached the coast near the outlet of River Thjorsa, south Iceland (63°50′N, 20°52′W) on 5 May 1990 and 3 May 1994. On 20 and 27 May 1994, additional birds were obtained from the Red Knot staging area at Selvogur, southwest Iceland (63°50′N, 21°43′W). Observations in southwest Iceland indicated that the 27 May sample was collected at the peak of the departures of Red Knots, as only few birds remained during the subsequent days; this confirms a departure schedule described before (Morrison and Wilson 1992). A reference sample of birds returning from the breeding grounds was collected at Selvogur on 17 July 1994. All birds were obtained by shooting.

On their high arctic breeding grounds, Red Knots were collected upon and after arrival in late May and early June 1987 in the vicinity of Alert (82°30′N, 62°20′W), which is situated on the northeast coast of Ellesmere Island, Northwest Territories, Canada. Usually fewer than 50 Red Knots were present around Alert, so it was possible to keep track of their phenology, e.g., the arrival of new flocks (Morrison and Davidson 1990). Birds collected on 30 and 31 May were recent arrivals, while those collected between 3 and 9 June were considered post-arrival "residents." An exception was made for one female collected on 5 June that was exceptionally heavy for that time, had a compositional profile typical of arriving birds, and had very small follicles. This individual is included in the "recent arrivals" sample.

Laboratory methods

Birds were processed immediately after collection (Ellesmere Island) or stored in freezers at temperatures of −20 to −30°C (Iceland). After transport to a Dutch laboratory, the birds from Iceland were weighed, measured, thawed, plucked, and dissected at organ level (Summers et al. 1992; Piersma et al. 1996). Three linear dimensions were taken. Wing length (flattened and stretched wing) was measured to the nearest millimetre with a stopped ruler, and bill length (exposed culmen) and the length of the sternum (dimension *a* in Piersma et al. 1984) were measured to the nearest 0.1 mm with calipers. Birds were sexed by gonadal inspection. All organs, the leg muscles, and the breast muscles were removed, after which the remaining skeleton with attached musculature was first dried to constant mass, then defatted in petroleum ether, and finally incinerated at 520–550°C. Note that the lower half of the tibia and the leg remained attached to the skin as it was removed. These pieces of bone are therefore not included in the incinerated portion of the bodies. The remaining ash was weighed to the nearest 0.01 g.

Birds collected on Ellesmere Island received different treatment. They were weighed and measured but not plucked and skinned. The heart, part of the liver, and stomach content were removed before the bodies were dried to constant mass. The dried carcasses were transported to the Netherlands, where fat was extracted and the dry, fat-free remainder was incinerated like the carcasses from Iceland.

Results

At both study sites and on all dates, the ash masses of males were lower than those of females (Table 1). The ash mass of males was rather stable over time, but that of females showed a steady increase in birds from Iceland during May and again in those from Ellesmere Island in late May – early June. This increase coincided with a steep increase in body mass that parallels the population average mass change in Iceland from about 140 g at arrival to about 210 g at departure (Wilson and Morrison 1992; personal observation). During their southward migration in Iceland, the ash mass of post-breeding adult females, but not males, was lower than their ash mass when they arrived from western Europe in spring (Table 1). On Ellesmere Island, Red Knots arrive with an average body mass of about 160 g, but subsequently both males and females lose some 30 g (personal observation). During these 10 days, at the end of which there is still about a week remaining before the beginning of egg laying, at least the females built up ash (Table 1).

Table 1. Sexual and seasonal variation in the ash content and body mass (averages only) of Red Knots collected in Iceland and on Ellesmere Island, Canada, in May–July.

	Iceland				Ellesmere Island	
	Arrival (3 and 5 May)	20 May	Departure (27 May)	Post breeding (17 July)	Arrival (late May)	Post arrival (early June)
Males						
Ash content (g)						
Average	3.75	3.62	3.64	3.54	4.27	4.54
SD	0.35	0.27	0.14	0.68	0.26	0.44
n	11	3	6	3	4	5
Body mass (g)	141	197	211	133	152	142
Females						
Ash content (g)						
Average	4.17	4.27	4.60	3.89	4.68	5.59
SD	0.66	0.09	0.46	0.12	0.60	0.47
n	8	2	6	2	2	8
Body mass (g)	136	207	218	138	152	132

Note: Values from birds from Iceland and Canada are not strictly comparable in view of differences in methodology (see the text).

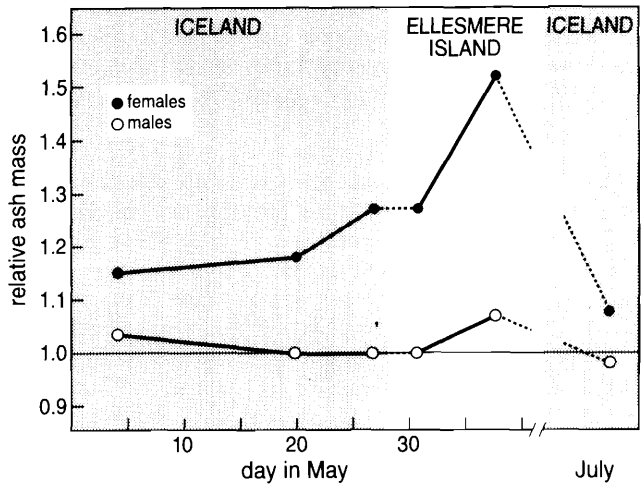
In view of the procedural differences in the handling of carcasses before ash values were obtained in a standardized way, separate series of covariance analyses were carried out on the data sets from the two study sites to see whether the sex differences and trends in averages reported in Table 1 were statistically significant and to determine the extent to which they reflect variation in body size (females are larger than males; e.g., Tomkovich 1992; Piersma et al. 1996). A series of covariance analyses of all data points from birds from Iceland, including different linear dimensions (wing, bill, and length of sternum) as covariates and sex and date as factors, consistently showed significant differences in ash mass between the sexes ($p < 0.01$), no effect of any of the three linear body dimensions on ash mass ($p > 0.7$), no interaction between sex and date ($p > 0.4$), but also no significant effect of date only ($p < 0.3$). For the birds collected on Ellesmere Island, covariance analyses were also unable to demonstrate significant effects of body size ($p > 0.5$), but showed significant effects of the factors sex ($p < 0.05$) and date ($p < 0.05$), even though the interaction of the factors did not reach significance ($0.1 < p < 0.3$). Interestingly, for both groups of birds covariance analyses in which body mass rather than ash mass was entered as the dependent variable showed no effects of sex but significant effects of the linear body dimensions instead. This further indicates that the difference in ash mass between males and females represents a real sex-related rather than a size-related dichotomy.

Discussion

That the ash mass of female Red Knots was consistently higher than that of males, even after statistical correction for the effect of body size, strongly suggests that the skeleton (which contributed most to ash mass) of females is relatively heavier than that of males. This was especially so in the week before the eggs were laid on the tundra of northern Ellesmere Island (Table 1). Also, the absolute difference between ash mass of males and females was smallest after the breeding season, during the birds' stopover in Iceland in July. At that point, any calcium stores should be absent and any difference only reflect the slight sexual dimorphism in body size. That the consistent increase in average ash mass of females in May was not statistically significant appears to be due to the fact that our sample sizes were small relative to the variation in the data. The increase in ash mass in the first week after arrival on the breeding grounds was significant, but occurred in both males (+0.27 g) and females (+0.91 g). Part of this increase is due to birds filling their guts, although the stomach contents of the Red Knots collected at the same time of the year in 1986 suggest a decrease rather than an increase in the amount of gravel and other indigestible matter with time after arrival (R.I.G.M., personal observation). No calcareous material was encountered in the stomach contents examined.

Nevertheless, the significant difference between ash masses of males and females, the stable ash mass of males over time, the consistent increase in ash mass of females before egg formation in mid-June, and the apparent subsequent depletion, according to the ash mass of females collected during the postbreeding return migration (Table 1), lead us to propose that female Red Knots do store skeletal calcium before egg laying. The evidence for this hypothesis is summarized in Fig. 1, where the relative temporal changes in ash mass of males and females are shown. We have scaled all averages to the mean ash mass of males collected just before departure from Iceland, and have assumed that no ash is lost during the 2900-km flight between southwest Iceland and northern Ellesmere Island. To determine the difference in calcium storage rates at the two sites, we have divided the best estimate of the daily relative increase in ash mass over 7 days on Ellesmere Island (the increase in males is subtracted from the increase in females to account for possible changes in the inorganic content of the gut) by the average relative increase over 23 days in Iceland. This calculation shows that

Fig. 1. Relative changes in ash content of Red Knots collected in Iceland and on Ellesmere Island, Canada, in May–July. This is based on the average values presented in Table 1, scaled to the average ash mass of males in Iceland and under the assumption that no ash mass was lost during the flight from Iceland to high arctic breeding areas such as Ellesmere Island.



the rate of calcium storage appears to be 2.3 times higher after arrival on Ellesmere Island than during the stopover in Iceland.

That female Red Knots should store most calcium after having completed the long flight to their high arctic breeding grounds makes sense if avian migrants try to minimize body mass at the start of long-distance flights. The extensive reorganisation between different functional organ groups that takes place in the bodies of Red Knots in Iceland strongly suggests that such mass minimisation is indeed practised (T. Piersma, G.A. Gudmundsson, and K. Lilliendahl, in preparation). On the other hand, the availability of calcium is certainly considerable in the Icelandic stopover areas, where Red Knots feed on calcium-rich molluscs in the intertidal zone (Alerstam et al. 1992). Snails and mussels are ingested whole and the shells are crushed in the muscular gizzard (Piersma et al. 1993). In view of the absence of calcareous material in the stomachs of the Red Knots collected on Ellesmere Island, we remain uncertain about the ways in which birds feeding on the tundra and the human sewage outflow near Alert obtain the calcium that is stored before egg formation in mid-June.

On the basis of the values presented by Graveland (1995), a shorebird species like the Red Knot, with a body mass of about 150 g and a predicted skeletal mass of 7.2 g, would need to double skeletal mass in order to have enough calcium ready to form four complete eggshells. It is clear that female Red Knots, which store about 2.5% of the average male's skeletal mass per day on the tundra (Fig. 1), are unlikely to achieve this, even if storage continues for a few more days after we sampled them. But with a 30–50% increase in skeletal mass (depending on whether the ash mass upon arrival in Iceland of males or of females is taken as a base line), Red Knots would be able to produce the eggshells of about half their clutch without further intake. Skeletal calcium depletion (e.g., Greeley 1962; Graveland 1995) could add another eggshell. Thus, once female Red Knots start producing their clutch of four, they could, in principle, rely to a considerable extent on their skeleton. Other free-living birds are able to release a maximum of about 20% of their skeletal calcium during egg formation (Graveland 1995, p. 86). Therefore, the suggested 50% change in skeletal mass shown by female Red Knots (Fig. 1), which probably reflects a similar proportional change in calcium content, makes the Red Knot the current avian record holder with respect to skeletal calcium dynamics.

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